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STRUCTURAL AND BIOPHYSICAL CHARACTERISATION OF
A GLUTAMATE RECEPTOR

by

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United States Army

EUROPEAN RESEARCH OFFICE OF THE U.S. ARMY

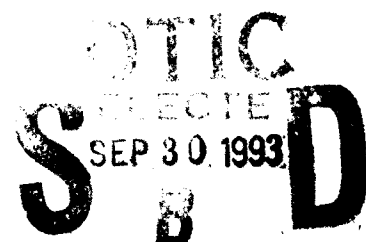
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11th Periodic & Final Report

The objectives of the proposed programme of research were as follows:

1. To isolate and purify glutamate receptors (GluR) of locust muscle.
2. To identify and to enrich the GluR mRNA using *Xenopus* oocytes.
3. To clone the cDNAs encoding the GluR, making use of peptide sequences to be determined on the purified molecule (for DNA probes) and of the appropriate mRNA fraction (for library construction).
4. To incorporate the purified GluR and the cloned GluR in lipid bilayers and, thereby to study more extensively the equilibrium and non-equilibrium kinetics of channel gating (and ligand binding) by the GluR.
5. Fully to characterise the structure of the GluR.

The immediate objectives of this work, namely to isolate, purify, structurally characterise and clone insect muscle GluR were not realised, mainly because of unforeseen technical difficulties, some of which have yet to be overcome. However, unexpectedly rapid developments in the molecular biology of mammalian GluR has reduced the need to use insect GluR as models for this class of receptor. We have taken advantage of these developments by designing new molecular biological procedures for over-expressing cloned mammalian GluR subunits. Since the long-term aim of this project is to produce biosensors based upon GluR, the availability of pure *mammalian* GluR is clearly advantageous in this respect.

Technical Material not previously reported

1. *Over-expression of the mammalian GluR1 subunit.*

Truncated and full-length cDNAs for GluR1 have been subcloned into the plasmid pGEM7 for over-production of the polypeptides encoded by these nucleotides. These cDNAs were subcloned in both sense and antisense orientations into the Eco RI site of pT7-7. A large amount of plasmid was subsequently isolated. The plasmid will now be introduced into the *E. coli* strain HMS174 for over-expression.

2. *Membrane-spanning peptides.*

Peptides of the M2, putative membrane-spanning regions of locust GluR Loc1-3 (Usherwood *et al.*, 1993) and the mammalian GluR1 and NR1 subunits have been synthesised (details in previous report). However, minute amounts of impurities in these synthetic peptides have subsequently been discovered and must be removed if studies involving the emplacement of the peptides in lipid bilayers are to provide unequivocal information. It is possible that the emplacement data that we have already obtained with the impure peptides represent fully the activities of the peptides in question, but this cannot be guaranteed. Unfortunately, the peptides concerned are difficult to purify to 100% because of their "sticky" nature. For this reason we have collaborated with Professor K. Nakanishi, Department of Chemistry, Columbia University, USA to develop new high-performance liquid chromatography system for purifying the M2 peptides. This collaboration has been highly successful (Lerro *et al.*, 1993) and mg quantities of the highly purified peptides are now available for emplacement in bilayers.

3. *Development of affinity columns using polyamine amide toxins to isolate GluR from mammalian brain.*

We have developed affinity columns for isolation and purification of GluR of mammalian brain using restrained polyamine amides based on either philanthotoxin-343 or argiotoxin-636. The interactions of these toxins with GluR of rat brain have been thoroughly investigated in our laboratory using electrophysiological procedures (Brackley *et al.*, 1993). Protein has been eluted from these columns with either L-kainic acid or N-methyl-D-aspartic acid and has been reconstituted into bilayers. Electrophysiological data from the bilayers has confirmed the presence of GluR (Volkova *et al.*, 1993). Current experiments are designed to evaluate the purity of the GluR fractions and to develop methods for increasing the yields of these proteins. This is part of an alternative strategy for obtaining mammalian GluR for biosensor development.

List of participating scientific personnel

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- (i) Professor P.N.R. Usherwood
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- (iii) Mr P. Brackley

Will graduate with the Degree of PhD in July 1993, which has been earned whilst working on the project.

(iv) Mr H. Sudan

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2. *Cambridge University*

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(i) Professor E.A. Barnard

(ii) Dr. R. Brindle

Publications emanating from the project

1. Usherwood, P.N.R. (1988). Insect glutamate receptors and their expression in *Xenopus* oocytes. Fourth ARO Workshop, Cashiers, N.Y.
2. Usherwood, P.N.R. (1992). From receptor proteins to peptides: a study of channel block. ARO Workshop, "Signal Transduction and Information Processing in Biological systems". Cashiers, N.Y.
3. Brackley, P.T.H., Bell, D.R., Choi, S.-K., Nakanishi, K. and Usherwood, P.N.R. (1993). Selective antagonism of native and cloned kainate and NMDA receptors by polyamine-containing toxins. *J. Pharm. Exp. Therap.* (In press).
4. Brackley, P.T.H. and Usherwood, P.N.R. (1993). Are chimeric kainate and N-methyl-D-aspartate receptors expressed in *Xenopus* oocytes from mammalian and amphibian RNA? *J. Pharm. Exp. Therap.* (In press).
5. Lerro, K.A., Orlando, R., Zhang, H., Usherwood, P.N.R. and Nakanishi, K. (1993). High-performance liquid chromatographic systems for the separation of sticky peptides from membrane proteins. (Submitted to *Biochemistry*).
6. Kerry, C. J., Sudan, H. L., Abutidze, K., Mellor, I.A., Barnard, E. A. and Usherwood, P.N.R. (1993). Reconstitution into artificial bilayers of glutamate receptor proteins purified from *Xenopus* central nervous system. *Mol. Pharm.* (In press).

7. Usherwood, P.N.R., Mellor, I., Breedon, L., Harvey, R.J., Barnard, E.A. and Darlison, M.G. (1993). Channels formed by M2 peptides of a putative glutamate receptor subunit of locust. In "Comparative Molecular pharmacology". Ed Pichon, Y., Birkhauser Verlag, Basel, Switzerland.
8. Breedon, L., Choi, S. -K., Mellor, I., Nakanishi, K. and Usherwood, P.N.R. (1993). The effect of philanthotoxin-343 on nicotinic acetylcholine receptors in lipid bilayers. (In preparation).
9. Volkova, T., Abutidze, K., Barnard, E.A., Kerry, C.J., Sudan, H.L. and Usherwood, P.N.R. (1993). Reconstitution of protein isolated from bovine and porcine brain. (In preparation).

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